Mechanistic Considerations in Benzene Physiological Model Development

Michele A. Medinsky, Elaina M. Kenyon, Mark J. Seaton, and Paul M. Schlosser

Chemical Industry Institute of Toxicology, Research Triangle Park, North Carolina

Benzene, an important industrial solvent, is also present in unleaded gasoline and cigarette smoke. The hematotoxic effects of benzene in humans are well documented and include aplastic anemia, pancytopenia, and acute myelogenous leukemia. However, the risks of leukemia at low exposure concentrations have not been established. A combination of metabolites (hydroquinone and phenol, for example) may be necessary to duplicate the hematotoxic effect of benzene, perhaps due in part to the synergistic effect of phenol on myeloperoxidase-mediated oxidation of hydroquinone to the reactive metabolite benzoquinone. Because benzene and its hydroxylated metabolites (phenol, hydroquinone, and catechol) are substrates for the same cytochrome P450 enzymes, competitive interactions among the metabolites are possible. *In vivo* data on metabolite formation by mice exposed to various benzene concentrations are consistent with competitive inhibition of phenol oxidation by benzene. *In vitro* studies of the metabolic oxidation of benzene, phenol, and hydroquinone are consistent with the mechanism of competitive interaction among the metabolites. The dosimetry of benzene and its metabolites in the target tissue, bone marrow, depends on the balance of activation processes such as enzymatic oxidation and deactivation processes such as conjugation and excretion. Phenol, the primary benzene metabolite, can undergo both oxidation and conjugation. Thus the potential exists for competition among various enzymes for phenol. Zonal localization of phase I and phase II enzymes in various regions of the liver acinus also impacts this competition. Biologically based dosimetry models that incorporate the important determinants of benzene flux, including interactions with other chemicals, will enable prediction of target tissue doses of benzene and metabolites at low exposure concentrations relevant for humans. — Environ Health Perspect 104(Suppl 6):1399–1404 (1996)

Key words: benzene, phenol, hydroquinone, human metabolism, physiological modeling

Introduction

Benzene is a ubiquitous industrial and environmental pollutant (*1*). It is present in automobile emissions, both evaporative and combustive, has been detected in cigarette smoke (*2*), and is commonly used as an industrial solvent in the workplace. Benzene is hematotoxic and carcinogenic at high concentrations. Epidemiologic studies have shown that people develop blood disorders such as pancytopenia, aplastic anemia, and acute myelogenous leukemia following repeated exposure to high concentrations of benzene (*3,4*). Benzene is also carcinogenic in animals (*5,6*).

Several studies have suggested that metabolism is a key step in the development of hematotoxicity (*7–9*). One current hypothesis suggests that several metabolic steps are required to produce the toxic metabolite (*10*). Following absorption in the blood and translocation to the liver, benzene is metabolized by cytochrome P450 (CYP) 2E1 to its major metabolite, phenol. Phenol can be further oxidized, most likely by the same CYP isozyme, to the polyhydroxylated metabolite, hydroquinone (*11,12*). Both phenol and hydroquinone are substrates for phase II enzymes such as sulfotransferases and glucuronyltransferases (*13*). Phenol and hydroquinone can also partition into blood and distribute to other tissues, including bone marrow. In bone marrow, the myeloperoxidase-dependent metabolism of hydroquinone to the reactive metabolite 1,4-benzoquinone is stimulated by the presence of phenol (*8*). 1,4-Benzoquinone is myelotoxic and clastogenic (*10,14*) and may be responsible for the toxic effects of benzene.

The multiple metabolic pathways for benzene provide opportunities for interactions that modulate benzene metabolism. Specific examples of these interactions and the net effect on the formation of benzene metabolites and resulting hematotoxicity or genotoxicity are discussed below.

Competitive Interactions between Benzene and Phenol

Studies in laboratory animals exposed to benzene by inhalation or oral administration have demonstrated that the metabolism of benzene is dose dependent (*15*). The metabolites of benzene are quantitatively excreted in urine, and the profile of urinary metabolites after benzene exposure can serve as an effective indicator of flux through the various metabolic pathways because little benzene is metabolized to CO_2 (*16*). In mice exposed to benzene by inhalation, the percentage of the total amount of urinary metabolites eliminated as hydroquinone glucuronide and hydroquinone sulfate decreased as the benzene exposure concentration increased from 5 to 600 ppm (*17*). In contrast, the glucuronide, sulfate, and glutathione conjugates of phenol represent an increasing percentage of the total metabolites eliminated in urine with increasing exposure concentration.

This dose-dependent metabolism of benzene can be explained in part by the interaction between benzene and its major metabolite, phenol. For example, both benzene and phenol are substrates for CYP2E1 (*11,12*) and have similar affinities. Because the concentrations of phenol in liver are much less than those of benzene, benzene can be an effective inhibitor of phenol oxidation, thereby decreasing the formation of hydroquinone conjugates.

This paper was presented at Benzene '95: An International Conference on the Toxicity, Carcinogenesis, and Epidemiology of Benzene held 17–20 June 1995 in Piscataway, New Jersey. Manuscript received 16 January 1996; manuscript accepted 14 June 1996.

Research described here was supported in part through a grant from the American Petroleum Institute and through an individual National Research Service Award (grant ES05630) awarded to E.M.K. by the National Institute of Environmental Health Sciences. We appreciate the comments of our colleagues, B. Kuyper, R. Cattley, C. Mugford, and P. Ammann.

E.M. Kenyon is now at the Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC.

Address correspondence to Dr. M.A. Medinsky, Chemical Industry Institute of Toxicology, 6 Davis Drive, PO Box 12137, Research Triangle Park, NC 27709-1237. Telephone: (919) 558-1250. Fax: (919) 558-1300. E-mail: medinsky@ciit.org

Abbreviations used: CYP, cytochrome P450; GI, gastrointestinal; HL, human liver; PV, portal vein; THV, terminal hepatic venule.

Blood concentrations of benzene in male F344 rats are 10-fold higher than those of phenol after a 6-hr inhalation exposure to 500 ppm, suggesting that there is sufficient benzene in the liver to inhibit phenol oxidation (*18*). With oxidation inhibited by benzene, phenol can be removed by enzymatic conjugation with glucuronic acid or phosphoadenosine–phosphosulfate. This mechanism of competitive inhibition is consistent with the decrease in total hydroquinone conjugates and the increase in phenol conjugates found with exposure to increasing doses of benzene (*17*). The decrease in hydroquinone glucuronide formation is especially apparent between 50 and 600 ppm, when metabolism shifts from apparent first-order to saturating. In fact, more total hydroquinone glucuronide is formed at 50 ppm than at 600 ppm (*17*). This unusual dose–response pattern is not explained by simple saturable or Michaelis-Menten kinetics but has been seen with other parent chemical/metabolite pairs that are substrates for the same enzyme (*19*).

Schlosser et al. (*11*) investigated the metabolism of benzene and phenol by mouse and rat liver microsome preparations *in vitro*. Using microsomal incubations, the conversion of benzene to its oxidation products can be studied apart from other detoxifying processes in a controlled and detailed manner. A quantitative simulation model describing the *in vitro* metabolism of benzene incorporated these reaction sequences: benzene→phenol and phenol→catechol→trihydroxy-benzene or phenol→hydroquinone→trihydroxybenzene. In the model, all reaction steps were assumed to be catalyzed by the same enzyme, CYP2E1. Benzene, phenol, hydroquinone, and catechol in solution were all assumed to compete through reversible binding for the same reaction site on CYP. The simulation model accurately described both benzene and phenol kinetic data supporting competition for a single enzyme. Figure 1 illustrates the formation of hydroquinone from phenol and the formation of hydroquinone from benzene using the *in vitro* simulation model for mice. The same rate constants and model structure were used to describe both data sets. The characteristic lag time for the formation of hydroquinone when starting with benzene as a substrate is due to the fact that phenol is an intermediate in the oxidation of benzene to hydroquinone. In particular, the simulation model suggests that the observed inhibitory effect of benzene on phenol metabolism and of phenol on benzene metabolism occurs through competition for a common reaction site that can also bind catechol and hydroquinone.

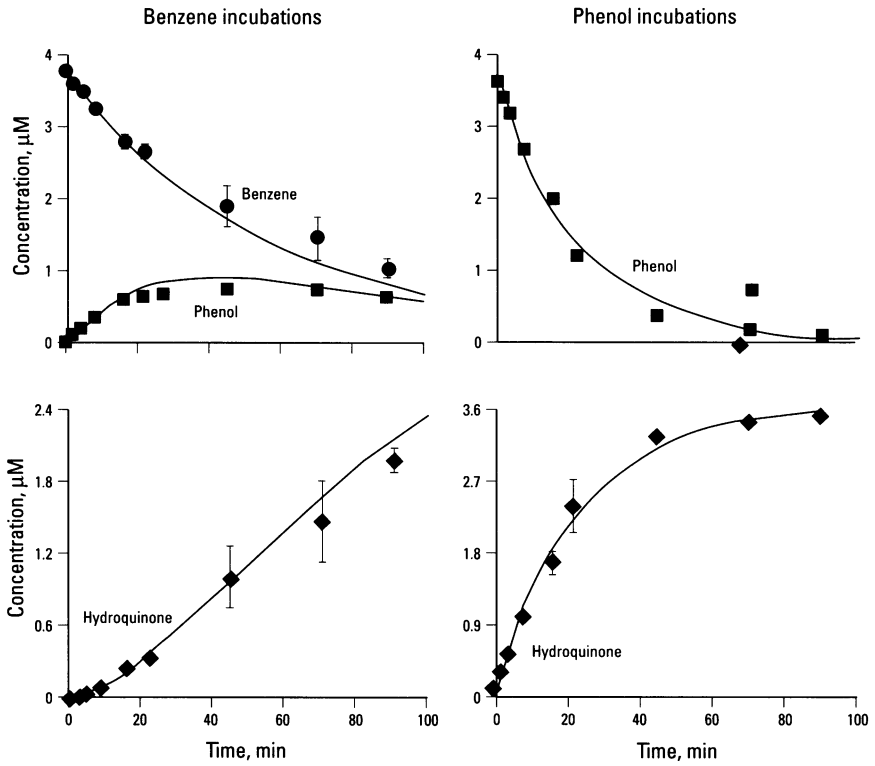

Figure 1. Data points and error bars (mean ± SD) are the results of experiments with mouse liver microsomes, and lines are model predictions for disappearance of substrate and formation of metabolites. Circles, squares and diamonds represent benzene, phenol, and hydroquinone, respectively. Solid lines are numerical simulations for the model equations using parameter values and model structure previously published by Schlosser et al. (*11*). Figure updated from Medinsky (*38*), with permission.

Heterogeneous Distribution of Hepatic Enzymes

Phenol plays a pivotal role in the metabolism of benzene because it is capable of undergoing oxidation to the polyhydroxylated metabolite hydroquinone and also conjugation via sulfo or glucuronyl transferases. Kenyon et al. (*20*) investigated the metabolism of phenol because of its importance as an oxidized metabolite of benzene and because of the distinct differences in both carcinogenic and genotoxic responses between benzene and phenol in mice. For example, although benzene is carcinogenic at multiple sites in mice after both oral and inhalation exposure (*5*), no increased incidence of tumors was found in a 2-year National Cancer Institute bioassay in which male and female B6C3F$_1$ mice were exposed to phenol in drinking water at levels of up to 5000 ppm (*21*). Benzene has been consistently found to induce micronuclei in mouse bone marrow cells (*22–24*). In contrast, both weakly positive and negative results have been reported for micronuclei induction in mouse bone marrow cells by phenol after ip and oral administration. Hydroquinone, the oxidized metabolite of phenol, induces sister chromatid exchanges *in vitro* in human lymphocytes (*14*) and *in vivo* in mouse bone marrow cells (*25,26*).

Insights into a possible metabolism-based explanation for the high potency of benzene as a carcinogen and the lack of genotoxic and carcinogenic effects of phenol can be gained by comparing urinary metabolite profiles after comparable oral doses of benzene and phenol. This comparison is presented in Table 1 using data for urinary excretion of metabolites following oral gavage of phenol in male B6C3F$_1$ mice (*20*) and similar data following oral gavage with benzene (*15*). Differences in the excretion of hydroquinone glucuronide are marked, particularly at the lower dose (approximately 1 mg/kg). Excretion of hydroquinone glucuronide in urine was 6-fold and 2-fold greater after benzene exposure compared

Table 1. Comparison of percentage of urinary metabolites excreted as phenol sulfate, phenol glucuronide, and hydroquinone glucuronide after gavage administration of phenol or benzene in male B6C3F$_1$ mice.

Chemical administered	Dose, mg/kg	Percentage of total metabolites in urine, mean±SE		
		Phenol sulfate	Phenol glucuronide	Hydroquinone glucuronide
Benzene[a]	1	26 ± 0.3	4.2 ± 0.3	43 ± 0.8
Phenol[b]	1.4	56 ± 0.9	39 ± 1.1	7.2 ± 0.4
Benzene[a]	10	32 ± 0.8	5.9 ± 0.3	36 ± 0.4
Phenol[b]	9.4	57 ± 7.7	26 ± 6.4	15 ± 1.6

[a]Urinary metabolite data after benzene exposure are from Sabourin et al. (*15*). [b]Urinary metabolite data following phenol exposure are from Kenyon et al. (*20*).

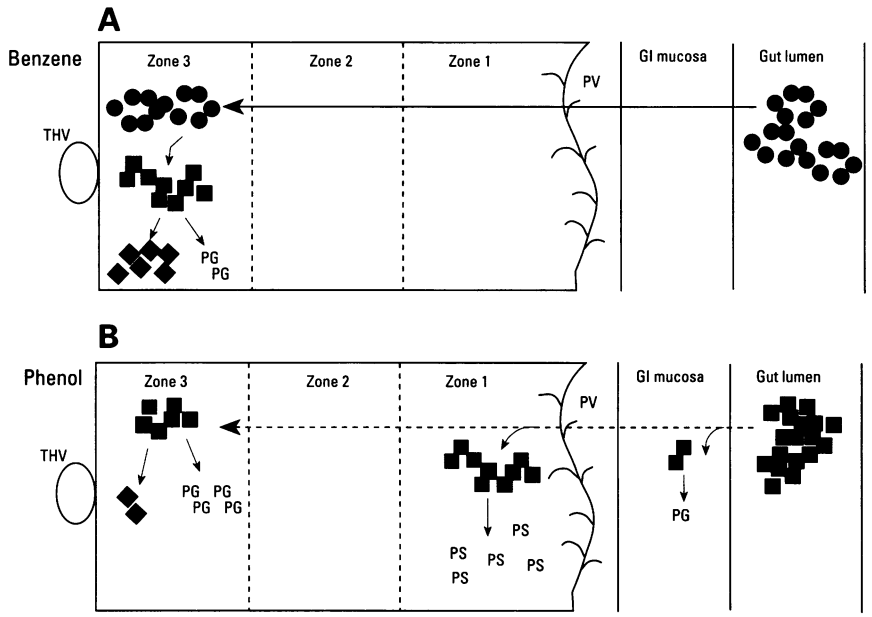

Figure 2. Hypothesized major relative hepatic zonal differences in metabolism of (*A*) benzene compared to (*B*) phenol after gavage administration during an initial pass through the liver. (●) Benzene, (■) phenol, (◆) hydroquinone. PV, portal vein; THV, terminal hepatic venule. Benzene and phenol absorbed from the gastrointestinal (GI) tract are sequentially available for metabolism in the GI mucosa and periportal (zone 1) and pericentral (zone 3) regions of the liver. The overall capacity of GI mucosal metabolism is low relative to that of the liver. Periportal localization of the enzymes (sulfotransferases) responsible for phenol sulfation suggests that less free phenol reached the pericentral hepatocytes, whereas higher levels of CYP2E1 activity are localized. The necessity for oxidation of benzene before conjugation suggests that more benzene will reach the pericentral hepatocytes for oxidation to phenol and hydroquinone. Figure adapted from Kenyon et al. (*20*), with permission.

with phenol at doses of approximately 1 and 10 mg/kg, respectively. This difference is of interest because hydroquinone is a potent inducer of micronuclei and chromosome aberrations. Thus, the greater production of hydroquinone after benzene administration compared with phenol administration, together with production of other toxic metabolites unique to benzene such as muconaldehyde, provides a possible metabolic basis for the carcinogenic and genotoxic effects observed with benzene but not with phenol.

The differences in the urinary metabolite profiles of phenol compared with benzene after oral administration are explicable in terms of quantitative differences in the zonal distribution of metabolizing enzymes within the liver acinus, as illustrated in Figure 2. Phenol absorbed from the gut lumen has a relatively greater opportunity for conjugation than for oxidation. Conjugation can occur initially in the gastrointestinal mucosal cells (sulfation and glucuronidation) and subsequently in periportal hepatocytes of zone 1 (sulfation) after phenol is absorbed into the portal circulation. Preferential conjugation of phenol is a consequence of a greater abundance of conjugating enzymes compared with oxidative enzymes in the periportal area, where phenol is distributed when initially absorbed (*27,28*). The net effect of this hypothetical scenario is that relatively little phenol reaches hepatic zone 3 where oxidative enzyme activity is greatest. Therefore, less hydroquinone is produced. In contrast to phenol, benzene requires oxidation by CYP2E1 (*12*) before it can be a substrate for conjugation, and CYP2E1 activity is much greater in the pericentral hepatocytes of zone 3 (*29*). Thus, metabolism of benzene is minimal in periportal and midzonal hepatocytes before reaching hepatocytes in zone 3, where oxidation and glucuronidation capacities are greater (*27,28*). Consequently, administration of benzene may result in the delivery of more free phenol to zone 3 oxidative enzymes than does administration of phenol itself.

The implications of these hypothetical scenarios are reflected in urinary metabolite profiles represented in Table 1 (*20*). Phenol sulfate excretion is approximately 2-fold higher and phenol glucuronide excretion approximately 4- to 9-fold higher after phenol compared with benzene, whereas oxidation products such as hydroquinone are higher after benzene exposure. The results of these studies suggest that first-pass intestinal conjugation of phenol in combination with zonal localization of hepatic enzymes contributes to the greater production of hydroquinone after oral administration of benzene compared with oral administration of phenol.

The concept that the fate of a metabolite derived from a precursor differs from that of a preformed metabolite is not new but was proposed by Pang and Gillette (*30*) for drugs. The kinetics of a drug and its metabolite can differ due to differences in diffusional barriers between the drug and hepatocytes, the rate of transport of the drug between hepatocytes, and the distribution of drug-metabolizing enzyme systems within the liver parenchyma (*31*). Using the drug-metabolite pair phenacetin and acetaminophen, Pang and colleagues observed differences in clearance of the chemicals from an isolated perfused liver depending on whether perfusion was conducted in a normal (portal to centrilobular) or retrograde (centrilobular to portal) direction (*28,30,31*). They attributed these differences to localization of P450 *O*-demethylation enzymes and sulfation enzymes in different zones of the liver. Pang and colleagues suggested that existing models of well-stirred livers were oversimplifications and could only be used in limiting cases when the distribution of enzymes was viewed as operationally uniform.

Using a combination of both experimental and mathematical modeling approaches, Pang et al. determined that substrate concentration at any point is influenced by preceding events during the metabolic processing of a substrate along the length of the liver sinusoid. Enzyme systems such as sulfation, when present upstream along the sinusoidal flow path, modify residual substrate available for metabolic processes such as oxidation within downstream hepatocytes. In the case of benzene and phenol, the residual phenol available for oxidation in the centrilobular region may be low when phenol is administered directly because most of it is conjugated upstream of this region. In contrast, when benzene is administered, the phenol is produced in the centrilobular region and thus is more available for subsequent oxidation to hydroquinone.

Smith et al. hypothesized that 1,4-benzoquinone is responsible for benzene-induced hematotoxicity (*10*). 1,4-Benzoquinone is formed from the myeloperoxidase-mediated oxidation of hydroquinone in the bone marrow. The net effect of heterogeneous distribution of enzymes in the liver and the resulting sequential oxidation of benzene and conjugation of phenol is higher blood levels of hydroquinone after benzene administration compared with phenol administration and more hydroquinone in bone marrow available for oxidation to 1,4-benzoquinone. Localization of enzymes in the liver provides explanations for differences both in observed toxicity and in *in vitro* metabolism of benzene and phenol. Thus, both the location and quantities of benzene-metabolizing enzymes must be incorporated into physiological models to predict risks for humans exposed to benzene.

The role of 1,4-benzoquinone in benzene-induced toxicity is brought into question by studies of McDonald et al., who observed high background concentrations of 1,4-benzoquinone adducts in globin isolated from human blood samples and in globin and bone marrow proteins from mice and rats (*32–34*). Additionally, the adducts in rodents exposed to benzene by gavage were only a small fraction of the steady-state 1,4-benzoquinone adduct levels. McDonald and coworkers attributed the source of the adducts to endogenous and dietary sources of phenol and hydroquinone. The fact that benzene exposure does not add significantly to the endogenous levels of these adducts suggests that these adducts and, by implication, 1,4-benzoquinone, do not play a role in benzene-induced toxicity. However, it must be recognized that background adduct levels result from long-term exposure to quinols, whereas the benzene-derived adducts observed in these studies were formed over a much shorter time. Thus, the rate of adduct formation from benzene-derived hydroquinone may greatly exceed the rate of formation from other sources. It may be the rate of adduct formation that is a true measure of risk. These results also point to the need for incorporating all sources of quinols (diet, cigarette smoking, and endogenous production) into a risk assessment for benzene if a quinol-related compound is used as the ultimate dosimeter.

Individual Variability in Human Metabolism of Benzene

The role of CYP2E1 in benzene metabolism was explored by Seaton et al., who compared biotransformation by liver samples from 10 humans as well as from mice and rats (Figure 3) (*35*). Benzene metabolism correlated strongly with CYP2E1 activity across species and individuals. A mathematical model was developed in which CYP2E1 activity was the only difference between individuals or species (*35*). This model also assumed that a single enzyme was responsible for oxidation, with benzene, phenol, and hydroquinone competing for the enzymatic reaction site. Comparing model predictions with experimental data suggests that the model does a fairly good job of predicting data to which it has not been fit (Figure 3).

Phenol sulfation and hydroquinone glucuronidation are the primary routes for conjugation and detoxication of benzene metabolites (*15*). The circulating levels of the oxidized metabolites are determined by the balance between oxidative rates and conjugation rates. Differences in the *in vitro* rates of conjugation between species or individuals were examined in pooled samples of rat and mouse liver and individual samples of human liver (*13*). The rate of phenol sulfate formation varied 3-fold among human samples (range, 0.3–0.9 nmol/mg/min) (Table 2). For laboratory animals, phenol sulfation was much faster in rat cytosol (1.2 nmol/mg/min) than in mouse cytosol (0.5 nmol/mg/min). Hydroquinone glucuronidation varied by almost 3-fold among human samples (range, 0.1–0.3 nmol/mg/min) (Table 2) and was more rapid in mouse liver microsomes (0.22 nmol/mg/min) compared with rat liver microsomes (0.08 nmol/mg/min). No correlation was observed among the rates of oxidation, sulfation, and glucuronidation for rats, mice, or individual humans.

Given the range of activities for oxidation, sulfation, and glucuronidation indicated in Table 2, what phenol and hydroquinone blood levels would occur during benzene exposure? A physiological compartmental model was developed to predict steady-state blood concentrations of phenol and hydroquinone that might be achieved during continuous exposure of rodents and humans to 0.01 μM benzene in blood (*13*). This blood concentration is likely to result from exposure to benzene at

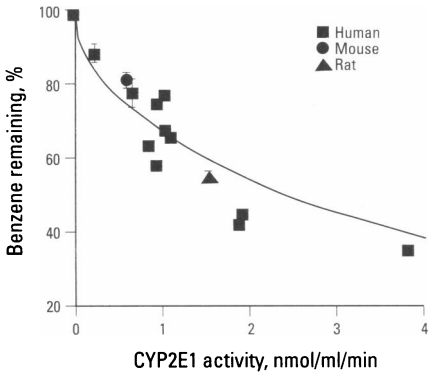

Figure 3. Metabolism of benzene by humans, rats, and mice, shown as a function of CYP2E1 activity. Data and model (solid line) are adapted from Seaton et al. (*35*).

Table 2. Initial rates for benzene oxidation, phenol sulfation, and hydroquinone glucuronidation in five human liver (HL) samples and single samples from mice and rats.[a]

Sample	Benzene oxidation, nmol/mg/min	Phenol sulfation, nmol/mg/min	Hydroquinone glucuronidation, nmol/mg/min
HL 1	0.344	0.309	ND
HL 2	0.926	0.581	0.117
HL 6	1.433	0.867	0.281
HL 7	1.499	0.919	0.167
HL 10	4.442	0.485	0.106
Mouse	1.558	0.485	0.218
Rat	0.625	1.195	0.077

ND, not determined due to insufficient sample. [a]Results from Seaton et al. (*13*).

the current 1 ppm Occupational Safety and Health Administration permissible exposure limit (*2*).

The predicted steady-state phenol and hydroquinone concentrations for the individual humans, rats, and mice are depicted in Figure 4. According to model calculations, predicted steady-state phenol concentrations varied 6-fold among humans and correlated inversely with measured microsomal activity of CYP2E1. This inverse relationship is most likely due to the fact that CYP2E1 is also responsible for phenol oxidation to hydroquinone in this model. While the rate of phenol formation increases with CYP2E1 activity, the rate of removal also increases with CYP2E1 activity, resulting in a net decrease in concentration. Steady-state hydroquinone concentrations varied 5-fold among humans. With respect to laboratory animals, model simulations predicted that during exposure to this low benzene dose, steady-state concentrations of phenol and hydroquinone would be higher in mice than in rats. These predictions are in agreement with *in vivo* observations from studies with higher benzene concentrations (*15*). Predicted steady-state concentrations in mice were higher than in humans, whereas values for rats fell among the range of predictions for humans. These simulations suggest that the rat may be a good model for humans with respect to tissue dosimetry of benzene metabolites.

Higher levels of oxidized metabolites in mice are due in part to the faster blood flow per unit body weight in this small species compared with humans. More benzene is brought to the liver for metabolism per unit of time. The model predictions for higher concentrations of oxidized metabolites in mouse blood also suggest that development of leukemia in humans is a function of tissue response in addition to dosimetry. The model predicts levels of the leukemogenic benzene metabolites in mice that are higher than those predicted for humans, and yet these levels do not give rise to acute myelogenous leukemia in mice. If these model predictions are correct, mouse bone marrow apparently responds to these agents in a manner that is qualitatively different from that in humans.

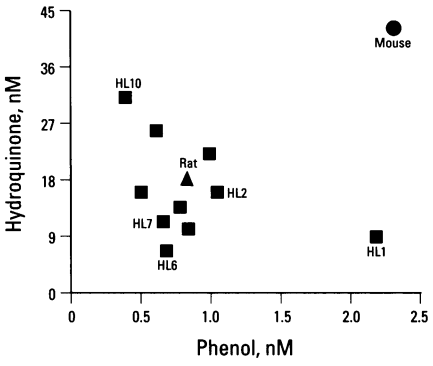

Figure 4. Predicted steady-state blood concentrations of phenol and hydroquinone for 10 individual humans, $B6C3F_1$ mice, and F344 rats when the blood concentration of benzene was constant at 0.01 μM. This blood concentration is approximately what would be expected for a continuous exposure to 1 ppm benzene. The steady-state phenol and hydroquinone concentrations are calculated from *in vitro* oxidation and conjugation activities using the physiological compartmental model reported by Seaton et al. (*13*). Figure adapted from Schlosser et al. (*39*), with permission.

Variability in rates of conjugation plays a definite role in determining steady-state blood concentrations of phenol and hydroquinone. Although the *in vitro* rate constants for benzene oxidation vary 13-fold among human samples, the steady-state concentration of phenol in blood vary only 6-fold. Moreover, steady-state concentrations of hydroquinone do not correlate with rates of oxidation reactions and appear to be influenced more by competition between phenol sulfation and phenol oxidation and detoxication due to hydroquinone glucuronidation. While the effect of the hydroquinone:phenol concentration ratio in blood on susceptibility to benzene toxicity is not clear, a synergistic relationship between these metabolites has been demonstrated *in vitro* (*7,10,36,37*).

The results described above suggest a significant interaction between phase I (oxidation) and phase II (conjugation) pathways in determining blood and tissue levels of benzene metabolites. The quality of these predictions should be tested in laboratory animals *in vivo* before using them for human risk assessment. Once this has been done, a model like that described here, taken together with the observed ranges of enzyme activities among humans, can be used to predict risk to individual humans with given enzyme activity profiles. Subsequently, the range of risk to human populations can be assessed.

Conclusions

The multiple metabolic pathways for benzene provide opportunities for modulation of benzene metabolism through competition between benzene and its metabolites, competition among the various enzymes for the substrate, and interactions of the metabolites in producing the toxicant. Specific examples of these interactions and the net effect on the formation of the toxic benzene metabolites have been discussed.

The dose-dependent metabolism observed for benzene is most likely the result of competition between benzene and phenol for the same oxidative enzymes. The complex dose–response relationship observed between benzene exposure and metabolite formation suggests that mathematical formulations of this mechanism must be incorporated into models designed to predict the dosimetry of benzene metabolites. The greater fraction of the administered dose metabolized to hydroquinone after gavage with benzene compared with phenol can be explained by the heterogeneous distribution of phase I and phase II enzymes in the liver. Heterogeneous enzyme distribution may explain why benzene exposure results in genotoxicity and carcinogenicity, while phenol exposure does not, even though a common metabolite, hydroquinone, has been implicated in the toxicity of benzene.

Because phenol and hydroquinone are components of a normal diet and may also arise through normal metabolic processes, the contribution of exogenous and endogenous sources of these two chemicals must be considered in estimating the total tissue dose of hydroquinone and the rate of genotoxic and cytotoxic damage. Incorporation of competitive interactions between substrates, heterogeneous distribution of hepatic enzymes, and synergistic interactions between metabolites into biologically based models of benzene should result in simulations of toxic metabolites in the bone marrow that are more predictive of observed toxicity. Ultimately, it should be possible to use biologically based model predictions of bone marrow dosimetry to predict risks for humans exposed to low concentrations of benzene, including the combination of model predictions for benzene metabolites derived from both endogenous and exogenous sources.

REFERENCES

1. Runion, HE, Scott, LM. Benzene exposure in the United States 1978-1983: an overview. Am J Ind Med 7:385–393 (1985).
2. Wallace L. Major sources of exposure to benzene and other volatile organic chemicals. Risk Anal 10:59–64 (1990).
3. Goldstein, BD. Hematotoxicity in humans. J Toxicol Environ Health Suppl 2:69–105 (1977).

4. Rinsky RA, Smith,AB, Hornung R, Filloon TG, Young RJ, Okun AH, Landrigan PJ. Benzene and leukemia, and epidemiological risk assessment. N Engl J Med 316:1044–1050 (1987).
5. Seaton MJ, Medinsky MA. Experimental studies of benzene carcinogenicity. Update on benzene. In: The Books of Medicine and Rehabilitative Medicine (Marcello I, ed), in press.
6. Snyder R, Kalf GF. A perspective on benzene leukemogenesis. Crit Rev Toxicol 24:177–209 (1994).
7. Irons RD, Stillman WS, Colagiovanni DB, Henry VA. Synergistic action of benzene metabolite hydroquinone on myelopoietic stimulating activity of granulocyte/macrophage colony-stimulating *in vitro*. Proc Natl Acad Sci USA 89:3691–3695 (1992).
8. Eastmond DA, Smith MT, Irons RD. An interaction of benzene metabolites reproduces the myelotoxicity observed with benzene. Toxicol Appl Pharmacol 91: 85–95 (1987).
9. Barale R, Marrazzini A, Betti C, Vangelisti V, Loprieno N, Barrai I. Genotoxicity of two metabolites of benzene: phenol and hydroquinone show strong synergistic effects *in vivo*. Mutat Res 244:15–20 (1990).
10. Smith MT, Yager JW, Steinmetz KL, Eastmond DA. Peroxidase-dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. Environ Health Perspect 82:23–29 (1989).
11. Schlosser PM, Bond JA, Medinsky MA. Benzene and phenol metabolism by mouse and rat liver microsomes. Carcinogenesis 14:2477–2486 (1993).
12. Koop DR, Laethem CL, Schnier GG. Identification of ethanol-inducible P450 isozyme 3a (P450IIE1) as a benzene and phenol hydroxylase. Toxicol Appl Pharmacol 98:278–288 (1989).
13. Seaton MJ, Schlosser PM, Bond JA, Medinsky MA. *In vitro* conjugation of benzene metabolites by human liver: potential influence of interindividual variability on benzene toxicity. Carcinogenesis 16:1519–1527 (1995).
14. Erexson GL, Wilmer JL, Kligerman AD. Sister chromatid exchange induction in human lymphocytes exposed to benzene and its metabolites *in vitro*. Cancer Res 45:2471–2477 (1985).
15. Sabourin PJ, Bechtold WE, Griffith WC, Birnbaum LS, Lucier G, Henderson RF. Effect of exposure concentration, exposure rate and route of administration on metabolism of benzene by F344 rats and B6C3F1 mice. Toxicol Appl Pharmacol 99:421–444 (1989).
16. Sabourin PJ, Chen BT, Lucier G, Birnbaum LS, Fisher, E, Henderson RF. Effect of dose on the absorption and excretion of ^{14}C-benzene administered orally or by inhalation in rats and mice. Toxicol Appl Pharmacol 87:325–336 (1987)
17. Medinsky MA, Sabourin PJ, Lucier G, Birnbaum LS, Henderson RF. A physiological model for simulation of benzene metabolism by rats and mice. Toxicol Appl Pharmacol 99:193–206 (1989).
18. Rickert DE, Baker TS, Bus JS, Barrow CS, Irons RD. Benzene disposition in the rat after exposure by inhalation. Toxicol Appl Pharmacol 49:417–423 (1979).
19. Clewell HJ, Andersen ME, MacNaughton MG. Inhalation pharmacokinetics: inhibitory interactions between *n*-hexane, methyl-*n*-butylketone, and 2,5-hexanedione. Toxicologist 4:111 (1984).
20. Kenyon EM, Seeley ME, Janszen D, Medinsky MA. Dose, route and sex-dependent urinary excretion of phenol metabolites in B6C3F1 mice. J Toxicol Environ Health 44:219–233 (1995).
21. NCI. Bioassay of Phenol for Possible Carcinogenicity. Technical Report Series NCI-CG-TR-203. Bethesda, MD:National Cancer Institute, 1980.
22. Gad-El Karim MM, Harper BL, Legator MS. Modifications in the myeloclastogenic effect of benzene in mice with toluene, phenobarbital, 3-methylcholanthrene, Aroclor 1254 and SKF-525A. Mutat Res 135:225–243 (1984).
23. Choy WN, MacGregor JT, Shelby MD, Maronpot RR. Induction of micronuclei by benzene in B6C3F1 mice: retrospective analysis of peripheral blood smears from the NTP carcinogenesis bioassay. Mutat Res.143:55–59 (1985).
24. Luke CA, Tice RR, Drew RT. The effect of exposure regimen and duration on benzene-induced bone-marrow damage in mice. I. Sex comparison in DBA/2 mice. Mutat Res 203:251-271 (1988).
25. Ciranni R, Barale R, Ghelardini G, Loprieno N. Benzene and the genotoxicity of its metabolites. II: The effect of the route of administration on the micronuclei and bone marrow depression in mouse bone marrow cells. Mutat Res 209:23-28 (1988).
26. Ciranni R, Barale R, Marrazzini A, Loprieno N. Benzene and the genotoxicity of its metabolites. I: Transplacental activity in mouse fetuses and in their dams. Mutat Res 208:61-67 (1988).
27. Gebhardt R. Metabolic zonation of the liver: regulation and implications for liver function. Pharmac Ther 53:275-354 (1992).
28. Morris ME, Pang KS. Competition between two enzymes for substrate removal in liver: modulating effects due to substrate recruitment of hepatocyte activity. J Pharmacokin Biopharm 15:473-496 (1987).
29. Tsutsumi M, Lasker JM, Shimizu M, Rosman AS, Lieber CS. The intralobular distribution of ethanol-inducible P450IIE1 in rat and human liver. Hepatology 10:437-446 (1989).
30. Pang KS, Gillette JR. Kinetics of metabolism and elimination in the perfused rat liver preparation: differences between the elimination of preformed acetominophen and acetominophen formed from phenacetin. J Pharmacol Exp Therap 207:178-194 (1978).
31. Pang KS, Terrell JA. Retrograde perfusion to probe the heterogeneous distribution of hepatic drug metabolizing enzymes in rats. J Pharmacol Exp Therap 216:339-346 (1981).
32. McDonald TA, Yeowell-O'Connell K, Rappaport SM. Comparison of protein adducts of benzene oxide and benzoquinone in the blood and bone marrow of rats and mice exposed to $^{14}C/^{13}C_6$]benzene. Cancer Res 54:4907–4914 (1994).
33. McDonald TA, Waidyanatha S, Rappaport SM. Production of benzoquinone adducts with hemoglobin and bone-marrow proteins following administration of [$^{13}C_6$]benzene to rats. Carcinogenesis 14:1921-1925 (1993).
34. McDonald TA, Waidyanatha S, Rapporport SM. Measurement of adducts of benzoquinone with hemoglobin and albumin. Carcinogenesis 14:1927–1932 (1993).
35. Seaton MJ, Schlosser PM, Bond JA, Medinsky MA. Benzene metabolism by human liver microsomes in relation to cytochrome P450 2E1 activity. Carcinogenesis 15:1799–1806 (1994).
36. Chapman DE, Namkung MJ, Juchau MR. Benzene and benzene metabolites as embryotoxic agents: effects on cultured rat embryos. Toxicol Appl Pharmacol 128:129–137 (1994).
37. Kolachana P, Subrahmanyam VV, Meyer K, Zhang L, Smith M. Benzene and its phenolic metabolites produce oxidative DNA damage in HL-60 cells *in vitro* and in the bone marrow *in vivo*. Cancer Res 53:1023–1026 (1993).
38. Medinsky MA. The application of physiologically-based pharmacokinetic/pharmacodynamic modeling to understanding the mechanism of action of hazardous substances. Toxicol Lett 79:185–191 (1995).
39. Schlosser PM, Kenyon EM, Seaton MJ, Medinsky MA. Determinants of benzene metabolism and disposition. CIIT Activities 15(6):1–10 (1995).